J Forensic Sci, January 2008, Vol. 53, No. 1
doi: 10.1111/j.1556-4029.2007.00599.x
Available online at: www.blackwell-synergy.com

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Identification of Colorants in Pigmented Pen Inks by Laser Desorption Mass Spectrometry*

ABSTRACT: Pigments are rapidly replacing dyes as colorants in pen and printer inks, due to their superior colors and stability. Unfortunately, tools commonly used in questioned document examination for analyzing pen inks, such as TLC, cannot be used for the analysis of insoluble pigments on paper. Laser desorption mass spectrometry is demonstrated here as a tool for analyzing pigment-based pen inks. A pulsed nitrogen laser can be focused onto a pen stroke from a pigmented ink pen on paper, and positive and negative ions representative of the pigment can be generated for subsequent mass spectrometric analysis. Targeted pens for this work were a set of Uni-ball® 207™ pigmented ink pens containing blue, light blue, orange, green, violet, red, pink, and black inks. Copper phthalocyanine was identified as the pigment used to make both blue inks. A mixture of halogenated copper phthalocyanines were identified in the green ink. Unexpectedly, the pink ink was found to contain a red pigment, Pigment Red 12, treated with a mixture of water-soluble dyes. Each sample yielded ions representative of the pigments present.

KEYWORDS: forensic science, questioned document examination, pigmented ink, laser desorption, mass spectrometry, colorants

In the field of questioned document analysis, the identification of the colorants in pen or printing inks on paper can be a useful investigative tool (1). On signed documents, checks, and handwritten items, the visible pen stroke is due to a colorant. In the case of blue ballpoint pens, the colorant has traditionally been a water-soluble dye, such as Crystal Violet, C.I. 42555 (2). The dyes are carried onto the paper in a vehicle, a solvent in which the dyes are soluble. When the dyes are the subject of analysis, they can be extracted from a portion of a writing sample and analyzed spectroscopically (e.g., UV–Vis spectroscopy) or chromatographically (TLC, HPLC) (1). While TLC is extensively used, the information it can provide (R_f values, number of dyes present and the color of each) is not molecular information, unless appropriate standards are identified.

Recently, pigmented inks have gained popularity both for pens and inkjet printers. Unlike dyes, pigments are much more stable, exhibit superior light-fastness, and are resistant to both photo- and chemical degradation (3). High molar absorptivities allow them to be used at very low concentrations, providing intense colors. Unlike dyes, pigments are not soluble in the vehicle but are suspended in it, as are pigments in a paint (3). One example of a pigment is copper phthalocyanine (Pigment Blue 15, C.I. 74160) which has been widely used to provide blue and black coloration since production began in the 1930s (4). Modification of this pigment, such as through chlorination, yields a green pigment (Pigment Green 7, C.I. 74260) with similar stability and intense color (3).

Manufacturers of pens that contain pigmented inks often highlight the benefit of their use in the context of the growing crime of check washing (5). While pen ink dyes can be washed from a check using common household cleaners or solvents, insoluble pigments remain on documents and cannot be washed away. This is an advantage to consumers, and a complication to document examiners. As pigments are insoluble, they cannot be removed from paper and cannot be separated by TLC or HPLC (1). The analysis of the two, dyes and pigments, are very different analytical challenges. The dyes exist as a thin layer of molecules on the substrate, while the pigments exist as solid particles on the substrate.

In the past several years, it has been shown that laser desorption mass spectrometry can be used for the direct analysis of colorants such as dyes from paper surfaces (2,6). Pen ink dyes such as crystal violet have been analyzed using this method (2). The ink-on-paper sample is introduced into the mass spectrometer, and the pen stroke is irradiated with a pulsed UV laser at 337 nm. These 2 nsec bursts of photons only interact with the dyes. Other components of the ink or paper do not absorb at this wavelength. The dyes absorb the UV light, and the resulting energy deposition leads to desorption/ionization of the dye molecules. Blue and red dyes have been studied using this method (2,6). Both anionic and cationic dyes respond, with those negatively-charged being detected in the negative ion mode of the MS and those positively-charged only detected in the positive ion experiment (2,6).

Here, a set of commercially available pigmented pen inks are characterized by LDMS. The method generates molecular information that can be used to identify the pigments in the pen inks. In some cases, the pigment particles appear to assist in the desorption/ionization of other components that do not, alone, absorb UV light. This additional information may be very useful in determining how long pigmented pen ink has been on a paper document.

Materials and Methods

The PE Biosystems Voyager DE time-of-flight mass spectrometer (Framingham, MA) is equipped with a pulsed nitrogen laser (337 nm) and a linear time-of-flight mass spectrometer. For the analysis of positive (negative) ions formed by LD, a sample plate, on which the analytes are placed, is held at 20,000 V (−15,000 V), an intermediate acceleration grid in the ion source is held at 94.5% of the accelerating voltage, and a delay time of 100 nsec was used between the laser irradiation pulse and initiation of ion acceleration. In a typical experiment, a pen-stroke was made on paper. A forensic punch (Harris-Uni-Core punch model 15074; Ted Pella, Inc.,

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*Presented at the 2006 Northeastern Association of Forensic Scientists Meeting, November 2, 2006, in Rye, NY.

Received 24 Feb. 2007; and in revised form 1 June 2007; accepted 5 June 2007.

Redding CA) was used to punch out a 1.25 mm diameter sample of the ink on paper, which was then secured onto a modified sample introduction plate (2,6) using double-stick tape (3M Scotch® Brand). All samples were applied to Boise Aspen™ white paper. For the analysis of the pink Uni-ball® 207™ pen ink, the TLC and paper chromatography solvent system was 90:50:36 (v:v:v) ethyl acetate:ethanol:water. The TLC plates used were Whatman Partisil® K6 (60 Å silica gel, 250 μm layer thickness). Bleaching of red dyes was achieved using Clorox® Regular Bleach. Separation of pink dyes and pigments was investigated using a Beckman Coulter Allegra® X-22 Centrifuge.

The LDMS instrument was calibrated using pen inks containing known dyes, applied to paper. Crystal violet, commonly found in blue ball point pen inks was used to calibrate in positive ion mode, while metanil yellow (commonly found with crystal violet in black ball point pen inks) was used in negative ion mode. Positive and negative ion spectra were generated, employing the parameters cited above. Under these conditions, the full resolution of the instrument is realized, even though ions are being formed from a nonconducting surface (paper) in the experiments.

Results and Discussion

The Uni-ball® 207™ pens are pigmented ink pens that are available in eight different colors (blue, light blue, green, orange, red, pink, purple, and black). They were chosen to develop methods for analyzing pigments from such pens by LDMS. For each, a pen stroke was made on paper. The pen stroke was sampled using a forensic document punch. This was introduced into the instrument and subjected to laser irradiation. Both positive and negative ions were formed, and mass spectra were obtained.

Blue

Many commercially available blue pigments exist, such as the triarylcarbonium Pigment Blue 1 (C.I.42595:2, $C_{33}H_{41}N_3$, MW 479), which is used to provide a wide range of brilliant shades (3). The positive and negative ion LDMS spectra of the blue pigmented ink are shown in Figs. 1*a* and 1*b*, respectively. In both spectra, there is an intense mass spectral peak at *m/z* 575, with a set of isotopic peaks at higher *m/z* values. The mass, isotope pattern, and pen ink color immediately resulted in the identification of the compound as the pigment copper phthalocyanine, which had been encountered previously (7,8). The structure is shown as the inset in Fig. 1*b*. The observed isotope pattern, which is shown as the inset in Fig. 1*a*, matches that predicted for the formula $C_{32}H_{16}N_8Cu$. The copper phthalocyanine molecule (M) forms both a molecular cation, M^+, and a molecular anion, M^-, in this experiment. This is to be expected for organic pigments and will be a key point to consider as spectra become more complex.

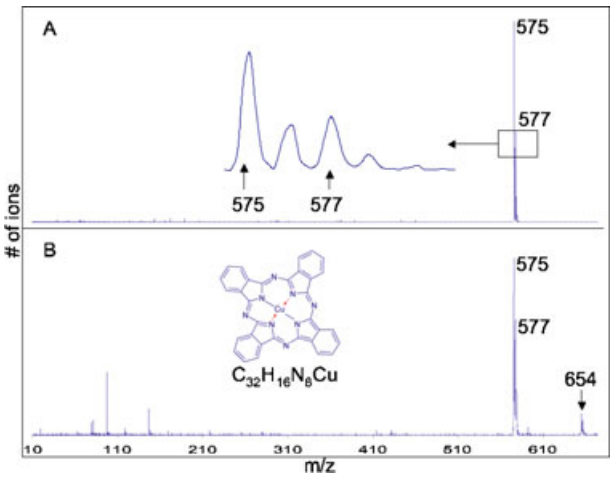

FIG. 1—*The positive and negative ion LDMS spectra of a pen stroke from a blue Uni-ball® 207™ pen on paper are shown in (a) and (b), respectively. The inset in (a) shows an expanded view of the isotopic peaks of the molecular ion. The structure of copper phthalocyanine is shown in the inset of (b).*

There are small, unidentified peaks observed in the negative ion spectrum (Fig. 1*b*) which are to be expected, as this is from a real ink sample which contains a variety of other compounds. In the negative ion spectrum, there is a peak at *m/z* 654, which is noteworthy. The peak is 79 Da above the M^- peak at *m/z* 575. This could represent the addition of bromine, with *m/z* 654 being an adduct ion of the neutral molecule and a bromide ion, $[M+Br]^-$. Another possibility is that a small amount of a modified copper phthalocyanine is present. If a H atom is replaced by an so_3^- group, the net increase in mass would be 79 Da as well. Thus the peak at *m/z* 654 could represent a monosulfonated copper phthalocyanine ion with the formula $C_{32}N_8H_{15}Cuso_3^-$. There are three reasons why this latter option is preferred. First, we have seen paints where there is a small amount of modified copper phthalocyanine present, added for stability (9). This may be the case here, with a sulfonated pigment added at low levels. Second, the isotope pattern observed, compared with that seen at *m/z* 575, would substantially change on addition of a Br atom, and would be very different than that for the copper phthalocyanine peak with sulfonate substitution. The isotope pattern of the *m/z* 654 peak matches that of the sulfonated ion, not the bromide adduct. Third, there is not a corresponding peak in the positive ion spectrum because this peak is not representing a neutral molecule, but an anionic derivative. Thus, it is only detected in the negative ion mode.

Light Blue

The Uni-ball® 207™ light blue pen results in the same spectra (not shown) as for the blue pen as shown in Figs. 1*a* and 1*b*. It therefore contains copper phthalocyanine as well, only at a lower concentration.

Green

An example of a green pigment often used is Pigment Green 1 (C.I. 42040:1, $C_{27}H_{33}N_2$, MW 385) (3). Different shades of this pigment can be achieved by mixing it with copper phthalocyanine; such blends are attractive choices as colorants due to their high tinctorial strength (3). The positive and negative LDMS mass spectra for the Uni-ball® 207™ green pigmented ink are shown in Figs. 2*a* and 2*b*, respectively. The spectra are very complex, containing many peaks above *m/z* 1000. This is unusual because most organic dyes and pigments, such as the example above of Pigment Green 1, have molecular weights below 800 g/mol. While the two spectra contain all peaks in common above *m/z* 1000, the most intense peaks in the negative ion spectrum are at relatively low *m/z* values.

As the *m/z* value increases in this experiment, the time-of-flight MS exhibits decreasing resolution. Above *m/z* 1000, it is usually difficult to completely resolve isotopic peaks, but they are still clearly seen. In the spectra shown, details of each peak show isotopic complexity. While the labeled peaks in the spectrum may each appear to be a single peak in the condensed version shown in the

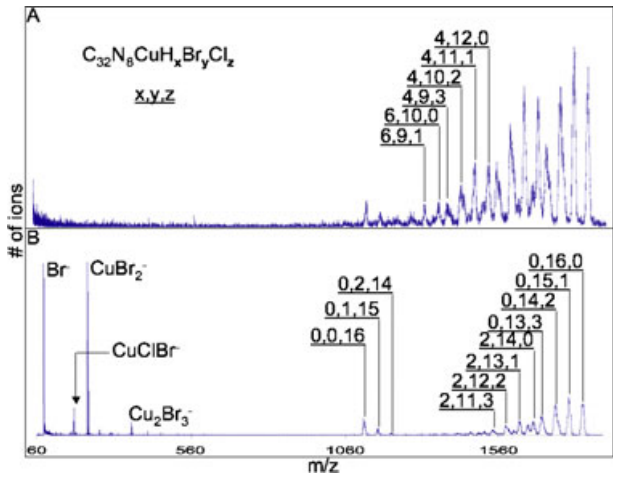

FIG. 2—*The positive and negative ion LDMS spectra of a pen stroke from a green Uni-ball® 207™ pen on paper are shown in (a) and (b), respectively. Peaks are labeled x, y, z to indicate the number of hydrogen, bromine, and chlorine atoms present in each copper phthalocyanine component.*

figure, they do have isotopic structure and are several Daltons wide.

One way to create a pigment with a high MW is to start with a large pigment molecule and replace H atoms with heavy atoms such as Cl, Br, or I, or large groups such as so_3^-. In the case of copper phthalocyanine, substituting the 16 H atoms with halogens shifts the color from blue to green, so it was not surprising to see peaks suggesting that the green pigment was a halogenated copper phthalocyanine.

As a reference point, the monoisotopic *m/z* value for copper phthalocyanine is 575. If all 16 H atoms are replaced by Cl atoms, the perchlorinated pigment ions would have a monoisotopic *m/z* value of 1119. If all 16 H atoms are replaced by Br atoms, the perbrominated pigment ions would have a monoisotopic *m/z* value of 1823. With the general formula $C_{32}N_8CuH_xBr_yCl_z$ for the halogenated forms, using the shorthand *x*, *y*, *z* to represent the number of H, Br, and Cl atoms, respectively, the perchlorinated 0, 0, 16 form and perbrominated 0, 16, 0 form are marked in Fig. 2. The series of peaks abruptly stops at 0, 16, 0. The isotopic distributions are consistent with theoretical distributions for molecules with these compositions. What are the other peaks? The first peak below the 0, 16, 0 peak is not 80 Da lower, indicating that it would be 1, 15, 0 but is instead about 44 Da lower. This is the difference between the mass of a bromine and a chlorine atom, thus the peak represents 0, 15, 1. Through similar analyses, the major peaks observed were identified as shown. Peaks appear at the same *m/z* values in the positive and negative ion spectra for each component of the mixture, consistent with what was observed for 16, 0, 0 in Fig. 1.

There remains the most intense peaks in the negative ion spectrum, as shown in Fig. 2*b*. The peaks are well-resolved and consist of a number of isotopic peaks, each separated by 2 Da. Chlorine, bromine, and copper are each elements that have abundant isotopes separated by 2 Da (^{35}Cl & ^{37}Cl, ^{79}Br & ^{81}Br, ^{63}Cu & ^{65}Cu). The assignments listed in Fig. 2*b* are consistent with the observed *m/z* values, number and relative intensities of the peaks representing the isotopic variants. For example, there are a set of peaks representing the ionic species $CuClBr^-$. The first peak, the monoisotopic peak, $^{63}Cu^{35}Cl^{79}Br^-$ has an *m/z* value of 177. Depending on whether the heavy or light form of each element is incorporated into the ion, the *m/z* value may range from 177 to 183—four peaks separated by 2 Da are expected with relative intensities of 57:100:51:8. Similarly, a set of four peaks from *m/z* 221–227 represent $CuBr_2^-$ and six peaks from *m/z* 363–373 represent the gas phase anion $Cu_2Br_3^-$. Apparently, when negatively-charged, the halogenated copper phthalocyanines fragment to generate these halogenated copper-containing anions. The spectra suggest that this does not occur for the cations. Presumably the addition of an electron destabilizes the bonding, making removal of the metal energetically accessible. A comparison of Figs. 1 and 2 shows that the analogous reaction does not occur for copper phthalocyanine—i.e., ions are not observed representing species such as CuH_2^-. This is understandable as C–H bonds are stronger than C–Br and C–Cl bonds in organic molecules (10).

The analysis presented here proved that the green pigment was a mixture of 20 or more halogenated copper phthalocyanines. What, then, is "the pigment"? There are a few halogenated copper phthalocyanines that are used commercially, and their chemical formulae are not well-defined. There is a pigment called Pigment Green 36. The CAS No. is 68425-85-4 and the Color Index No. is 742650. It is described by one pigment producer as 5, 9, 2 (11). Another source lists Pigment Green 36 as CAS No. 14302-13-7, C.I. No. 74265, with the formula 0, 6, 10 (12). Yet another manufacturer's web site lists the formula as 16, 6, 10 (13). More accurate, although more vague definitions of Pigment Green 36 include ranges. One reference states that it contains 4–5 chlorine atoms and 11–12 bromine atoms (14). Another states that it contains 4–9 bromine atoms and 8–2 chlorine atoms (3).

Pigment Green 7 (Cyanine Green G, C.I. 74260, CAS No. 1328-53-6), is reported to have the formula 2, 0, 14 (15). Another source suggests that Pigment Green 7 "contains *c.* 15 chlorine atoms" (16). Wikipedia states that Pigment Green 7 has a chemical formula ranging from 3, 0, 13 to 1, 0, 15 (17). It is clear that, in the business of dyes and pigments, there is often no single chemical compound associated with a pigment name but rather a manufacturing process, color, and set of physical properties. The mixtures can be complex, and in the case of a mixture of chloro-, bromopigments, which cannot be separated by standard chromatographic techniques, the LDMS method may provide the best approach for defining the distribution of components. In response to the question of what composes the pigment, it is surely not Pigment Green 7, which only contains chlorine atoms. The pigment in the pen ink is likely Pigment Green 36. Compounds over the full range from 0, 0, 16 to 0, 16, 0 are present. The spectra suggest an average MW for the pigment molecules of *c.* 1690 g/mol, which is somewhat higher than what most of the formulae would suggest. For example, the description of Pigment Green 36 as 0, 10, 6 leads to a MW of 1383 g/mol.

Orange

For many years, the most popular orange pigment was the inorganic compound lead chromate, $PbCrO_4$ (12). Now, modern organic pigments, such as *β*-Naphthol Pigment Orange 5 (C.I. 12075, $C_{16}H_{10}N_4O_5$, MW 338), are more often used (3). The positive and negative ion LDMS mass spectra of the orange pigmented ink are shown in Figs. 3*a* and 3*b*, respectively. The positive and negative ion spectra have some similarities but are noticeably different. While we may approach the interpretation by assuming that there is a mixture of pigments, the composition may be simpler. There is a set of peaks beginning at *m/z* 622 in the positive ion spectrum, which do not appear to represent a single species. The more complex distribution of isotopic peaks at *m/z* 622 is consistent with the formation of both M^+ and MH^+ ions, in a *c.* 1:2 ratio.

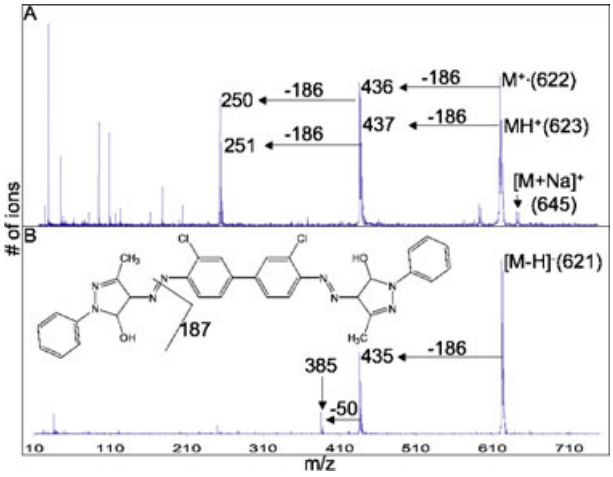

FIG. 3—*The positive and negative ion LDMS spectra of a pen stroke from an orange Uni-ball® 207™ pen on paper are shown in (a) and (b), respectively. Also shown is the structure of Pigment orange 13.*

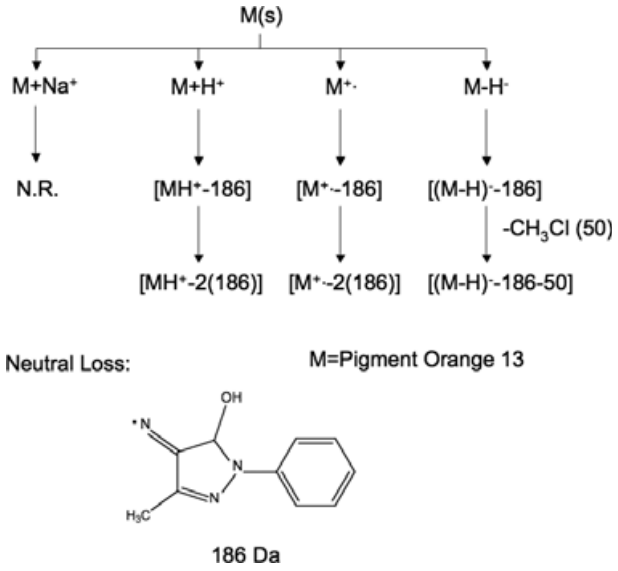

FIG. 4—*A summary of the ionic processes observed for ionized Pigment Orange 13 molecules.*

When there are sufficient salts in a system, it is also typical to see sodium ion or potassium ion adducts formed in the positive ion spectrum. There are a set of peaks beginning at *m/z* 645, 23 Da higher than the *m/z* 622 peak. By considering these two sets of peaks, we conclude the following: First, the peaks represent a compound with a MW of 622. The peak at *m/z* 645 is an $[M+Na]^+$ peak, which clearly shows from the isotopic distribution that the molecule contains two chlorine atoms. The molecular ion takes two forms, each with isotopic peaks representative of the molecular formula, overlapping in this region of the spectrum. The intense peaks at lower *m/z* values in Fig. 3*a* appear to represent fragment ions of the molecular ion, rather than additional pigments, as they do not have peaks 23 Da higher as does the peak for the intact molecule. For the molecule to fragment so extensively, the structure must be very different than that of copper phthalocyanine, and there must be skeletal bonds that can be easily broken following ionization. In the negative ion spectrum, the peaks beginning at *m/z* 621 represent the deprotonated molecules, $[M–H]^-$, and provide the same information regarding the presence of two Cl atoms.

With this minimal information—a MW of 622, an orange pigment with two chlorines, and a pigment capable of fragmenting following ionization—a candidate pigment was identified, Pigment Orange 13 (Pyrazolone Orange, C.I. 21110), $C_{32}H_{24}Cl_2N_8O_2$. Pigment Orange 13 ranks high among orange pigments used commercially (3).

A good test of the identity of the orange pigment with the MW of 622 is to determine if the lower *m/z* ions are in fact fragment ions and if they are consistent with the structure of Pigment Orange 13. In Fig. 3*b*, there is a peak at *m/z* 435, 186 Da lower than the $[M–H]^-$ peak. The *m/z* 435 peak has a set of isotopic peaks that are very similar to that for *m/z* 621—indicative of the presence of two chlorine atoms. The situation is more complicated in Fig. 3*a* because two forms of the molecular ion, M^+ and MH^+, are observed. Apparently, both the molecular ions and the protonated molecules fragment to lose a neutral with a mass of 186 Da as well, yielding peaks at 436 and 437 (with accompanying isotopes indicative of the retention of both chlorine atoms in the fragment ions). A second loss of 186 Da leads to the peaks at *m/z* 250 and 251, with both chlorines still retained in the ionic products.

The structure of Pigment Orange 13 is shown inset in Fig. 3*b*. If the N=N bond is broken, a neutral can be generated with a mass of 187 Da. As the neutral loss corresponds to a mass of 186 atomic mass units (amu), this indicates that the bond is broken with an accompanying H shift, from the departing neutral onto the fragment ion. While one may not expect the double bond to be a facile cleavage point, consider that ionization may weaken the bond, and at some point a H shift occurs accompanying the bond cleavage. Such reactions have been observed previously; for example, the azo pigment Pigment Yellow 83 has been shown to cleave at the N=N location of the molecule (18). Also, in a study of pigments in automotive paints by LDMS, N=N bonds in ionized benzimidazolone molecules (Benzimidazolone Orange 36), were observed to cleave with an accompanying H shift to yield a fragment ion (19). The identical group can be eliminated from either side, in the same way, with no impact on the chlorine content of the fragment ion. Thus, the spectra are consistent with the candidate pigment structure Pigment Orange 13. It is interesting to note that, while the M^+ and MH^+ ions eliminate two neutrals of 186 amu, fragmentation is less extensive for the $[M–H]^-$ ions, and only one loss of 186 amu occurs. However, there is a second loss. There is a peak 50 Da below the *m/z* 435 peak in Fig. 3*b*. The isotopic distribution changes, indicating that the neutral molecule lost with a mass of 50, contains one Cl. This corresponds to the elimination of CH_3Cl. The processes by which the major peaks in Fig. 3 are formed are summarized in Fig. 4. The pigment molecules are ionized in four ways. When Na^+ adducts are formed, the charge remains on the sodium atom, and no fragmentation follows. For the protonated molecules, protonation probably occurs on a nitrogen atom, initiating fragmentation and a H-shift. If the molecular cation is formed, the electron lost may well be a nonbonding electron on a nitrogen atom, again initiating fragmentation. When $[M–H]^-$ ions are formed, it is likely that conversion of an –OH group to $–O^-$ starts the fragmentation process.

Violet

Compounds such as the benzimidazolone Pigment Violet 32 (C.I. 12517, $C_{27}H_{24}N_6O_7S$, MW 576) are popular among violet pigments because of their broad range of applications and compatibility with various media (3). The positive and negative ion LDMS spectra of the violet pigmented ink on paper are shown in Figs. 5*a*

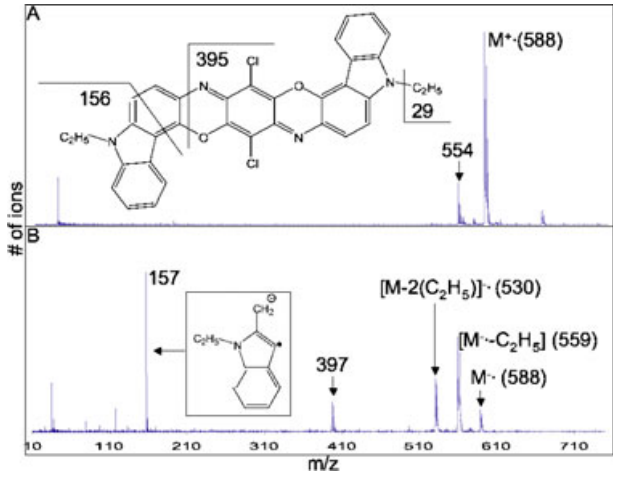

FIG. 5—*The positive and negative ion LDMS spectra of a pen stroke from a violet Uni-ball® 207™ pen on paper are shown in (a) and (b), respectively. Also shown is the structure of Pigment Violet 23.*

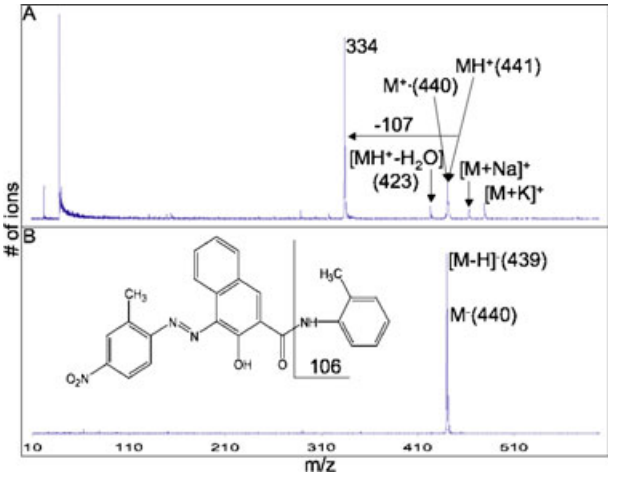

FIG. 6—*The positive and negative ion LDMS spectra of a pen stroke from a red Uni-ball® 207™ pen are shown in (a) and (b), respectively. Also shown is the structure of Pigment Red 12.*

and 5*b*, respectively. There is a peak at *m/z* 588 in both positive and negative ion spectra, with isotopes consistent with the presence of two chlorine atoms. The initial assumptions are that the monoisotopic mass is 588, and that this is a pigment that forms M^+ and M^-. In the positive ion spectrum shown in Fig. 5*a*, there is a peak at *m/z* 554, 34 Da lower, which could represent a monochlorinated form of the parent pigment.

The negative ion spectrum gives more chemical information on the pigment. Aside from the M^- peak at *m/z* 588 with its characteristic Cl isotope pattern, there also exist two lower mass peaks at *m/z* 559 and *m/z* 530 with a retention of the Cl isotope pattern of the intact molecular ion. These peaks are 29 and 58 Da lower than the M^- peak and signify the loss of one and two ethyl radicals, respectively. The presence of chlorine atoms "centrally located" in the molecule, and multiple losses of the same mass due to molecular symmetry, is similar to the situation encountered with the orange pigment discussed earlier. A search for a purple pigment with a MW of 588, two centrally located chlorine atoms, and cleavable ethyl groups, resulted in the identification of Pigment Violet 23, or Carbazole Violet (C.I. 51319), whose structure is shown in the inset of Fig. 5*a*. It is commercially used for "coatings and paints to plastics, printing inks, and other special purpose media" (3).

Two other peaks in Fig. 5*b* are consistent with this assignment. The peak observed at *m/z* 397 is assigned to the fragment ion resulting from the cleavage of the O–C and N–C linkages in the six-membered heterocycle of Pigment Violet 23. Once again, the Cl isotope pattern of the parent anion is retained in the fragment anion at *m/z* 397. However, this cleavage would form a fragment ion at *m/z* 395 while the peak appears at *m/z* 397; this indicates that the cleavage is accompanied by a shift of two hydrogens from the departing neutral onto the fragment ion. The most abundant fragment ion in Fig. 5*b* is *m/z* 157, which lacks the typical Cl isotope pattern. This indicates that an unusual cleavage occurs to produce an unlikely fragment ion without attached chlorines, and a neutral molecule with the two chlorines attached. Bond cleavage as shown in Fig. 5*a* with an accompanying H shift would yield a fragment ion with a mass of 157 amu. A structure for the fragment ion is shown in Fig. 5*b*.

Red

The *β*-Naphthol Pigment Red 3 (C.I. 12120, $C_{17}H_{13}N_3O_3$, MW 307), also known as Toluidine Red, is an example of a common red pigment, extensively used, that is "by volume one of the 20 largest organic pigments in the world" (3). The positive and negative ion LDMS mass spectra of the red pigmented ink are shown in Figs. 6*a* and 6*b*, respectively. There are four peaks in the *m/z* 430–500 range in the positive ion spectrum, but only one in the negative ion spectrum. As seen previously, such as in the orange ink spectra, it is not unusual to see alkali ion adducts of neutral molecules in positive ion spectra. The set of observed peaks suggest that there are four forms of the intact ionized molecule formed in the positive ion experiment—a molecular ion, protonated molecule, sodium ion adduct, and potassium ion adduct, consistent with a compound having a MW of 440. This is also suggested by the negative ion spectrum, where intense peaks at *m/z* 439 and 440 represent the M^- and $[M–H]^-$ ions. Isotopic peaks show that the red pigment contains no Cl or Br atoms. There are two known pigments that could satisfy these criteria—Pigment Red 12 or Pigment Red 17; the difference between the two pigments is only in the positioning of a nitro group relative to a methyl group. However, as Pigment Red 12, or Permanent Bordeaux F2R (C.I. 12385), has more commercial utility than Pigment Red 17 (C.I. 12390), it is selected as the identity of the pigment in the ink (3). Its structure is shown in Fig. 6*b*.

Two fragment ions are observed in the positive ion spectrum, at *m/z* 423 and 334. These should also be consistent with the proposed structure. The peak at *m/z* 423 corresponds to loss of water from the protonated molecule, and reflects the presence of an –OH group. The peak at *m/z* 334 corresponds to a neutral loss of 107 amu from the protonated molecule at *m/z* 441. The structure in Fig. 6*b* suggests a skeletal bond cleavage that would yield a neutral of mass 106. Loss of 107 amu would correspond to loss of this fragment with an accompanying H atom, possibly initiated by protonation of the amide nitrogen. Similar mechanisms are common for peptide sequencing by MS, where protonated peptides fragment by cleavage of such amide ("peptide") bonds (20). For these two cationic fragment ions, at *m/z* 423 and 334, the relative intensities are consistent with the fact that protonation would occur on an –NH group preferential to an –OH group as amines have higher proton affinities than alcohols.

Pink

As the spectra for the blue and light blue pigmented inks are the same, we anticipated that the spectra for the pink ink would match

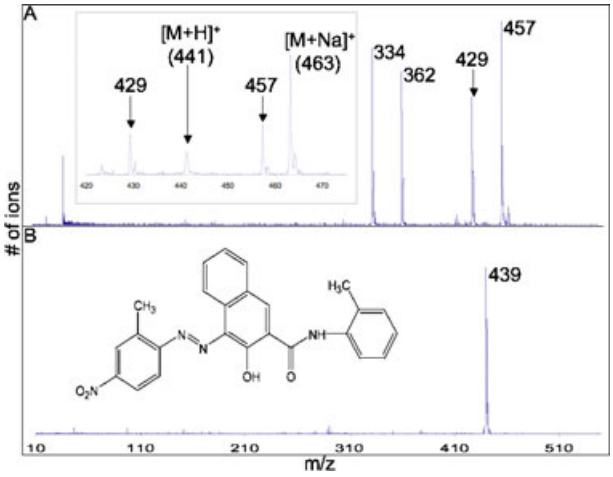

FIG. 7—*The positive and negative ion LDMS spectra or a pen stroke from a pink Uni-ball® 207™ pen on paper are shown in (a) and (b), respectively. Experimental results suggested that the pigment used also carried two dyes with it. When the dyes were partially bleached, the positive ion spectrum changed to that shown in the inset of (a). Also shown is the structure of Pigment Red 12.*

those for the red ink. The spectra are shown in Fig. 7*a* (positive) and Fig. 7*b* (negative). While the negative ion spectrum in Fig. 7*b* shows the anticipated $[M-H]^-$ peak for the red pigment at *m/z* 439, the positive ion spectrum in Fig. 7*a* does not display the corresponding protonated red pigment peak at *m/z* 441; instead, a set of new peaks is observed. As much of the spectra in Figs. 6 and 7 are similar, the discussion will focus on the portion that is different—the new peaks of interest at *m/z* 429 and *m/z* 457. A set of two peaks in the positive ion spectra at *m/z* 429 and 457, with the same isotopic peaks, had been observed previously in the study of red dyes used in security inks (21). They represented cationic dyes and thus only appear in the positive ion spectrum. While it was surprising to see the two together again, it was also unexpected to find dyes present in this pink pigmented ink.

A dot of pink pigmented ink was applied directly to a TLC plate. A sample of a dollar bill that had been stained with the red security ink from a previous study (21) was extracted and analyzed on the same TLC plate. The results showed that both samples contained the same two dyes, with matching R_f values. In solution, their UV–Vis spectra (not shown) and λ_{max} values matched as well. There was also a significant component of the pink ink that did not move in the TLC solvent, presumably representing a pigment. The conclusion was that the pink ink contains a pigment and at least two dyes. The *m/z* 439 peak in Fig. 7*b* suggested that Pigment Red 12 was used, as in the red ink, but why is there no corresponding peak in the positive ion spectrum, as observed in Fig. 6*a*? In other types of "desorption/ionization" mass spectrometry such as MALDI (matrix-assisted laser desorption/ionization) MS and FAB (fast atom bombardment) MS, there have been discussions in the literature of a process called suppression, where the presence of one analyte suppresses the detection of another (22). By "detection," we specifically mean formation of ions representative of an analyte. If there are cationic dyes present, or more specifically, if the pigment particles had been "dyed" with dye molecules, perhaps the positive dye ions suppress the formation of MH^+ ions from the pigment. To test this, and in pursuit of an experiment that would allow us to detect confirming ions in the positive ion spectrum for the presence of Pigment Red 12, several experiments were performed. First, some pigmented ink from the pink pen was washed, repeatedly, with methanol and water, and centrifuged. Each washing appeared to remove substantial amounts of dye, however when the centrifuged solid pigment was isolated following washings, it still showed the *m/z* 429 and 457 dye-related peaks, with no evidence for the presence of the pigment in the positive ion spectrum. The most conclusive result was obtained as follows: a line of pink pigmented ink was drawn on a piece of filter paper, which was then subjected to elution in a conventional paper chromatography experiment. Two bands resulted—the pigment band, that did not move, and the dye bands that moved together. There is no guarantee that all of the dyes were washed off the pigment. Bleaching of the dyes was considered. When Chlorox bleach was applied to the dye band, it became colorless, so bleach was applied to the pigment band. Presumably we now have pigment and a colorless form of the dyes, the leuco forms (4), in the same location on the paper. The paper was lightly washed to remove excess bleach and allowed to dry. A forensic punch was then used to sample the pigment on paper for LDMS analysis. The positive ion spectrum that resulted is shown as the inset in Fig. 7*a*. As shown, there are still peaks at *m/z* 429 and 457 representing the cationic dyes, now greatly reduced. In this experiment, positive ions at *m/z* 441 and *m/z* 463 now appear, representing the MH^+ and MNa^+ ions of an analyte pigment with a MW of 440. Thus, the pink pigmented ink did contain dyed pigment, and the cationic dyes appear to have been suppressing the detection of the pigment in the positive ion mode. It should be noted that there is a type of colorant called a lake, where colorless substrates are dyed and the product is used as would be a pigment. In this case, dye was applied to a pigment to yield the desired pink color. Perhaps the diluted red did not give a satisfactory pink, so a dye was used to brighten up the ink. While a dye-treated pigment, a "lake," (23) may be less than ideal for use in some fields such as cosmetics, it should be fine for a pen ink. Also we note that, if all of the pen inks used in this study are observed under UV light, only the pink fluoresces. Thus, the dyes did substantially influence the color of the ink—allowing it to be a "dilute red" but still vibrant.

Black

The LDMS analysis of the black Uni-ball® 207™ pigmented ink was one of the more difficult tasks due to the intrinsic nature of black pigments. They are often carbon-based. For example, lamp black is from the soot of burning coals, tar, crude naphthalene, creosote, and other petroleum products; ivory black is impure carbon obtained by charring animal bones. There are also black pigments that are inorganic, such as Pigment Black 11, (C.I. 77499, $FeO^-Fe_2O_3$, FW 232) (12). While organic pigments are relatively new to pen inks, black carbon powders as pigments have been used for many years in writing inks.

Figure 8*a* shows the typical positive ion LDMS spectrum obtained for the black ink on paper. No peaks are detected except for a peak at *m/z* 39 representing K^+ ions. The negative ion spectra that were obtained varied. They always contained only low *m/z* peaks, but could be complicated or relatively simple depending on where in the pen stroke the sample was taken. A typical spectrum is shown in Fig. 8*b*—many low *m/z* peaks are observed including a series of C_n^- ions such as C_4^- at *m/z* 48. When some locations where irradiated, the negative ion spectra were simpler, such as that shown in Fig. 8*c*. Here a set of peaks representing C_n^- ions is observed, from n = 2–13, clearly showing that the pigment is a carbon black.

By increasing the laser power, an additional set of peaks in the range of *m/z* 600–1500 could consistently be generated, as

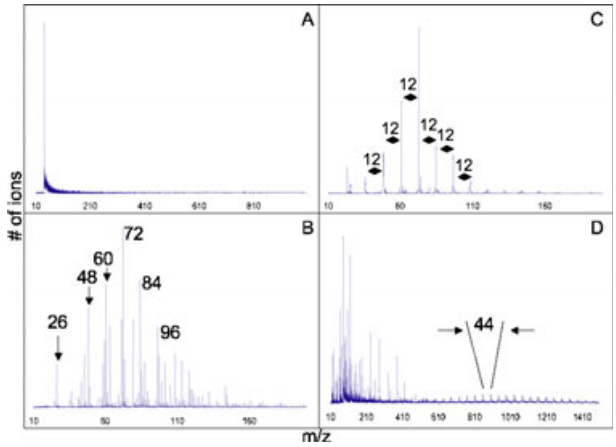

FIG. 8—*(a) Shows the positive ion spectrum of a pen stroke from a black Uni-ball® 207™ pen on paper. (b and c) Represent negative ion spectra collected from the same sample. If the laser power is increased, additional peaks at higher m/z values appear, as shown in (d).*

shown in Fig. 8*d*. With a distinctive separation of 44 amu, the peaks were quickly identified as a set of ions representing oligomers of polyethylene glycol (as $[(H–(C_2H_4O)_n–OH)–H]^-$ ions). Polyethylene glycols are commonly used as binders and thickening agents and are used in the formulation of pen inks (1). Polyethylene glycols do not absorb at 337 nm, so the results may suggest that the absorbing carbon particles can assist in the desorption/ionization of molecules adhered to the surface, in this case, oligomers (and in Fig. 8*b*, perhaps small absorbed molecules).

Conclusions

LDMS has been shown to be a useful and sensitive tool for the chemical identification of dyes, and now pigments that may be used as colorants in pen inks. As laser desorption can be performed directly from a paper substrate, there is no solubility requirement. As pigments cannot be analyzed by the commonly used method of TLC, this is an attractive option. While the degradation of simple pen ink dyes over time may be useful for estimating the age of a written sample, pigments do not appear to change over time. However, the detection of the polyethylene glycols was an encouraging result. In addition to the added feature of being able to detect nonabsorbing components in the presence of an absorbing pigment, preliminary work suggests that the intensities of the polyethylene glycol peaks decrease with time. This would be plausible, due to evaporation or migration into the paper away from the pen stroke. Perhaps such measurements may allow estimations to be made concerning the age of a document written using a pigmented ink.

Acknowledgments

The authors thank the National Institute of Justice for providing financial support for this work. Work presented here was supported under Award No. 2005-IJ-CX-K064 from the Office of Justice Programs, National Institute of Justice, Department of Justice.

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